EAST Search History

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	11	"5753008"	USPAT; EPO; DERWENT	AND	ON	2006/04/25 19:57
L2	21	"5049282"	USPAT; EPO; DERWENT	AND	ON	2006/04/25 18:21
L3	1	l1 and covalent	USPAT; EPO; DERWENT	AND	ON	2006/04/25 18:25
L4	5	2 and covalent	USPAT; EPO; DERWENT	AND	ON	2006/04/25 19:21
L5	1	1 and covalent bond	USPAT; EPO; DERWENT	AND	ON	2006/04/25 19:21
L6	15	diaminohexane same propanol and membrane	USPAT; EPO; DERWENT	AND	ON	2006/04/25 19:58
L7	12	6 and support	USPAT; EPO; DERWENT	AND	ON	2006/04/25 20:00
L8	0	210/500.37 and 6	USPAT; EPO; DERWENT	AND	ON	2006/04/25 20:01
L9	2	210/500.37 and diaminohexane	USPAT; EPO; DERWENT	AND	ON	2006/04/25 20:05
L10	0	9 and alcohol	USPAT; EPO; DERWENT	AND	ON	2006/04/25 20:02
L11	0	9 and covalent	USPAT; EPO; DERWENT	AND	ON	2006/04/25 20:04
L12	29	diaminohexane same alcohol and membrane	USPAT; EPO; DERWENT	AND	ON	2006/04/25 20:05
L13	3	12 and polyimide	USPAT; EPO; DERWENT	AND	ON	2006/04/25 20:05
L14	0	13 and covalent	USPAT; EPO; DERWENT	AND	ON	2006/04/25 20:06
L15	3	13 and heating	USPAT; EPO; DERWENT	AND	ON	2006/04/25 20:06

EAST Search History

L16	1	15 and support	USPAT; EPO; DERWENT	AND	ON	2006/04/25 20:12
L17	3	12 and composite	USPAT; EPO; DERWENT	AND	ON	2006/04/25 20:14
L18	0	210/500.38 and 12	USPAT; EPO; DERWENT	AND	ON	2006/04/25 20:15
L19	0	210/500.38 and diaminohexane same propanol	USPAT; EPO; DERWENT	AND	ON	2006/04/25 20:16
L20	0	210/490 and diaminohexane same propanol	USPAT; EPO; DERWENT	AND	ON	2006/04/25 20:16

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RCE

TITLE: Derivatization of solid **supports** and methods for oligomer synthesis

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APPL-NO: 08/956986

DATE FILED: October 24, 1997

PARENT-CASE:

RELATED APPLICATIONS

This application is a division of U.S. patent application Ser. No. 08/728,720, filed Oct. 10, 1996, now abandoned which is a division of U.S. patent application Ser. No. 08/431,196, filed Apr. 27, 1995, now U.S. Pat. No. 5,642,711.

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CIPS B01J19/00 20060101
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CIPS C03C17/34 20060101
CIPS C03C17/30 20060101
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FIELD-OF-CLASSIFICATION-SEARCH: 427/333; 427/340 ; 427/385.5
See application file for complete search history

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N/A

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ART-UNIT: 172

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ABSTRACT:

Methods and derivatized **supports** which are useful in solid-phase synthesis of peptides, oligonucleotides or other small organic molecules as well as arrays of ligands. The methods provide means to control the functional site density on a solid **support**. Some of the derivatized **supports** are polymer-coated or glycan-coated. Other methods for regenerating the surface of a used ligand array are also provided.

7 Claims, 17 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 17

---------- KWIC ---------

Abstract Text - ABTX (1):

Methods and derivatized **supports** which are useful in solid-phase synthesis of peptides, oligonucleotides or other small organic molecules as well as arrays of ligands. The methods provide means to control the functional site density on a solid **support**. Some of the derivatized **supports** are polymer-coated or glycan-coated. Other methods for regenerating the surface of a used ligand array are also provided.

TITLE - TI (1):

Derivatization of solid **supports** and methods for oligomer synthesis

Brief Summary Text - BSTX (2):

The present invention relates to the field of solid phase polymer synthesis. More specifically, the invention provides methods and derivatized **supports** which find application in solid phase synthesis of oligomer arrays or of single compounds on a preparative scale. The oligomer arrays which are prepared using the derivatized **supports** of the present invention may be used, for example, in screening studies for determination of binding affinity and in diagnostic applications.

Brief Summary Text - BSTX (3):

The synthesis of biological polymers such as peptides and oligonucleotides

has been evolving in dramatic fashion from the earliest stages of **solution** synthesis to solid phase synthesis of a single polymer to the more recent preparations of libraries having large numbers of diverse oligonucleotide sequences on a single solid **support** or chip.

Brief Summary Text - BSTX (9):
The present invention provides a variety of derivatized **supports** and methods for their preparation, which are useful in the preparation of peptides, oligonucleotides or other small organic molecules.

Brief Summary Text - BSTX (11):
A number of novel derivatized **supports** are provided which have altered surfaces, for example polymer-coated or glycan-coated solid **supports**. Other derivatized **supports** utilize linking groups terminating in acidic functionalities such as carboxylic acids or sulfonic acids which are useful in alternative synthesis strategies.

Brief Summary Text - BSTX (12):
The present invention further provides methods of rendering the derivatized **supports** reusable.

Brief Summary Text - BSTX (15):
In another aspect, the present invention provides methods for the preparation of stabilized polymer-coated **supports** for use in solid-phase synthesis. These methods typically use dip coating, **covalent** polymer attachment, in situ polymerization, or combinations thereof to provide the polymer-coated **support**.

Brief Summary Text - BSTX (16):
In yet another aspect, the present invention provides glycan-coated **supports** and methods for their preparation. While similar to the polymer-coated **supports,** the properties of glycan-coated **supports** can be quite different and provide extremely hydrophilic surfaces which are useful in binding assays and diagnostic applications.

Drawing Description Text - DRTX (4):
FIG. 3 provides the structures of three polymers (**polyethyleneimine,** polyacrylamide and polyallylamine) which are useful for preparing polymer-coated glass substrates.

Drawing Description Text - DRTX (5):
FIG. 4 provides the structures of a group of carboxylic acid and anhydride

polymers which are useful for the preparation of polymer-coated solid **supports**.

Drawing Description Text - DRTX (6):
FIG. 5 provides the structures of polyethylene glycol and poly(vinyl **alcohol**) which are useful for preparing polymer-coated solid **supports**.

Drawing Description Text - DRTX (7):
FIG. 6 is an illustration of a polymer-coated glass substrate which can be prepared by dip coating, **covalent** crosslinking or in situ polymerization.

Drawing Description Text - DRTX (8):
FIG. 7 illustrates one example of polymer crosslinking which uses glutaraldehyde and **polyethyleneimine**.

Drawing Description Text - DRTX (9):
FIGS. 8-11 illustrate a variety of methods for covalently attaching a polymer to a derivatized solid **support**.

Drawing Description Text - DRTX (10):
FIG. 12 illustrates in situ polymerization which can be used to prepare a polymer-coated solid **support**.

Detailed Description Text - DETX (16):
Chemical terms: As used herein, the term "alkyl" refers to a saturated hydrocarbon radical which may be straight-chain or branched-chain (for example, ethyl, isopropyl, t-amyl, or 2,5-dimethylhexyl). When "alkyl" or "alkylene" is used to refer to a linking group or a spacer, it is taken to be a group having two available valences for **covalent** attachment, for example, --CH.sub.2 CH.sub.2 --, --CH.sub.2 CH.sub.2 CH.sub.2 --, --CH.sub.2 CH.sub.2 CH(CH.sub.3)CH.sub.2 --and --CH.sub.2 (CH.sub.2 CH.sub.2).sub.2 CH.sub.2 --. Preferred alkyl groups as substituents are those containing 1 to 10 carbon atoms, with those containing 1 to 6 carbon atoms being particularly preferred. Preferred alkyl or alkylene groups as linking groups are those containing 1 to 20 carbon atoms, with those containing 3 to 6 carbon atoms being particularly preferred. The term "polyethylene glycol" is used to refer to those molecules which have repeating units of ethylene glycol, for example, hexaethylene glycol (HO--(CH.sub.2 CH.sub.2 O).sub.5 --CH.sub.2 CH.sub.2 OH). When the term "polyethylene glycol" is used to refer to linking groups and spacer groups, it would be understood by one of skill in the art that other polyethers or polyols could be used as well (i.e, polypropylene glycol or mixtures of ethylene and propylene glycols).

Detailed Description Text - DETX (19):

The term "activating agent" refers to those groups which, when attached to a particular functional group or reactive site, render that site more reactive toward **covalent** bond formation with a second functional group or reactive site. For example, the group of activating groups which are useful for a carboxylic acid include simple ester groups and anhydrides. The ester groups include alkyl, aryl and alkenyl esters and in particular such groups as 4-nitrophenyl, N-hydroxylsuccinimide and pentafluorophenol. Other activating agents are known to those of skill in the art.

Detailed Description Text - DETX (20):

Ligand: A ligand is a molecule that is recognized by a receptor. Examples of ligands which can be synthesized using the methods and compounds of this invention include, but are not restricted to, agonists and antagonists for cell **membrane** receptors, toxins and venoms, viral epitopes, hormones, opiates, steroids, peptides, enzyme substrates, cofactors, drugs, lectins, sugars, oligonucleotides, nucleic acids, oligosaccharides, and proteins.

Detailed Description Text - DETX (21):

Monomer: A monomer is a member of the set of small molecules which are or can be joined together to form a polymer or a compound composed of two or more members. The present invention is described herein in terms of compositions and methods which are useful in solid phase synthesis. In a number of applications, solid phase methods are used for the preparation of biological polymers such as peptides, proteins and nucleic acids. For these biological polymers, the set of monomers includes but is not restricted to, for example, the set of common L-amino acids, the set of D-amino acids, the set of synthetic and/or natural amino acids, the set of nucleotides and the set of pentoses and hexoses. The particular ordering of monomers within a biological polymer is referred to herein as the "sequence" of the polymer. As used herein, monomers refers to any member of a basis set for synthesis of a polymer. For example, dimers of the 20 naturally occurring L-amino acids form a basis set of 400 monomers for synthesis of polypeptides. Different basis sets of monomers may be used at successive steps in the synthesis of a polymer. Furthermore, each of the sets may include protected members which are modified after synthesis. The invention is described herein primarily with regard to the preparation of molecules containing sequences of monomers such as amino acids, but could readily be applied in the preparation of other polymers. Such polymers include, for example, both linear and cyclic polymers of nucleic acids, polysaccharides, phospholipids, and peptides having either .alpha.-, .beta.-, or .omega.-amino acids, heteropolymers in which a known drug is covalently bound to any of the above, polynucleotides, polyurethanes, polyesters,

polycarbonates, polyureas, polyamides, **polyethyleneimines,** polyarylene sulfides, polysiloxanes, **polyimides,** polyacetates, or other polymers which will be apparent upon review of this disclosure. Such polymers are "diverse" when polymers having different monomer sequences are formed at different predefined regions of a substrate. Methods of cyclization and polymer reversal of polymers are disclosed in copending application U.S. Ser. No. 08/351,058 which is a CIP of U.S. Ser. No. 07/978,940 which is a CIP of U.S. Pat. No. 5,242,974, entitled "POLYMER REVERSAL ON SOLID SURFACES," incorporated herein
by reference for all purposes.

Detailed Description Text - DETX (22):

In certain embodiments of the invention, polymer-coated **supports** are described. The polymers used for coating a solid **support** include, but are not limited to polyurethanes, polyesters, polycarbonates, polyureas, polyamides, **polyethyleneimines,** polyarylene sulfides, polysiloxanes, polyacrylamides, **polyimides,** polyacetates, or other polymers which will be apparent upon review of this disclosure. The polymers used to coat a solid **support** are typically repeats of a single monomers which is crosslinked with a second molecule to provide structural integrity to the polymer.

Detailed Description Text - DETX (24):

Receptor: A receptor is a molecule that has an affinity for a ligand. Receptors may be naturally-occurring or manmade molecules. They can be employed in their unaltered state or as aggregates with other species. Receptors may be attached, covalently or noncovalently, to a binding member, either directly or via a specific binding substance. Examples of receptors which can be employed by this invention include, but are not restricted to, antibodies, cell **membrane** receptors, monoclonal antibodies and antisera reactive with specific antigenic determinants, viruses, cells, drugs, polynucleotides, nucleic acids, peptides, cofactors, lectins, sugars, polysaccharides, cellular **membranes,** and organelles. Receptors are sometimes referred to in the art as anti-ligands. As the term receptors is used herein, no difference in meaning is intended. A "Ligand Receptor Pair" is formed when two molecules have combined through molecular recognition to form a complex.

Detailed Description Text - DETX (25):

Substrate: As used herein, the term "substrate" or "**support**" refers to a material having a rigid or semi-rigid surface. In many embodiments, at least one surface of the substrate will be substantially flat, although in some embodiments it may be desirable to physically separate synthesis regions for different polymers with, for example, wells, raised regions, etched trenches,

or the like. In some embodiments, the substrate itself contains wells, trenches, flow through regions, etc. which form all or part of the synthesis regions. According to other embodiments, small beads may be provided on the surface, and compounds synthesized thereon may be released upon completion of the synthesis.

Detailed Description Text - DETX (31):

"Light-directed" methods (which are one technique in a family of methods known as VLSIPS.TM. methods) are described in U.S. Pat. No. 5,143,854, previously incorporated by reference. The light directed methods discussed in the '854 patent involve activating predefined regions of a substrate or solid **support** and then contacting the substrate with a preselected monomer **solution**. The predefined regions can be activated with a light source, typically shown through a mask (much in the manner of photolithography techniques used in integrated circuit fabrication). Other regions of the substrate remain inactive because they are blocked by the mask from illumination and remain chemically protected. Thus, a light pattern defines which regions of the substrate react with a given monomer. By repeatedly activating different sets of predefined regions and contacting different monomer **solutions** with the substrate, a diverse array of polymers is produced on the substrate. Of course, other steps such as washing unreacted monomer **solution** from the substrate can be used as necessary.

Detailed Description Text - DETX (33):

Additional methods applicable to library synthesis on a single substrate are described in co-pending applications Ser. No. 07/980,523, filed Nov. 20, 1992, and Ser. No. 07/796,243, filed Nov. 22, 1991, incorporated herein by reference for all purposes. In the methods disclosed in these applications, reagents are delivered to the substrate by either (1) flowing within a channel defined on predefined regions or (2) "spotting" on predefined regions. However, other approaches, as well as combinations of spotting and flowing, may be employed. In each instance, certain activated regions of the substrate are mechanically separated from other regions when the monomer **solutions** are delivered to the various reaction sites.

Detailed Description Text - DETX (34):

A typical "flow channel" method applied to the compounds and libraries of the present invention can generally be described as follows. Diverse polymer sequences are synthesized at selected regions of a substrate or solid **support** by forming flow channels on a surface of the substrate through which appropriate reagents flow or in which appropriate reagents are placed. For example, assume a monomer "A" is to be bound to the substrate in a first group

of selected regions. If necessary, all or part of the surface of the substrate in all or a part of the selected regions is activated for binding by, for example, flowing appropriate reagents through all or some of the channels, or by washing the entire substrate with appropriate reagents. After placement of a channel block on the surface of the substrate, a reagent having the monomer A flows through or is placed in all or some of the channel(s). The channels provide fluid contact to the first selected regions, thereby binding the monomer A on the substrate directly or indirectly (via a spacer) in the first selected regions.

Detailed Description Text - DETX (37):

One of skill in the art will recognize that there are alternative methods of forming channels or otherwise protecting a portion of the surface of the substrate. For example, according to some embodiments, a protective coating such as a hydrophilic or hydrophobic coating (depending upon the nature of the solvent) is utilized over portions of the substrate to be protected, sometimes in combination with materials that facilitate wetting by the reactant **solution** in other regions. In this manner, the flowing **solutions** are further prevented from passing outside of their designated flow paths.

Detailed Description Text - DETX (38):

The "spotting" methods of preparing compounds and libraries of the present invention can be implemented in much the same manner as the flow channel methods. For example, a monomer A can be delivered to and coupled with a first group of reaction regions which have been appropriately activated. Thereafter, a monomer B can be delivered to and reacted with a second group of activated reaction regions. Unlike the flow channel embodiments described above, reactants are delivered by directly depositing (rather than flowing) relatively small quantities of them in selected regions. In some steps, of course, the entire substrate surface can be sprayed or otherwise coated with a **solution**. In preferred embodiments, a dispenser moves from region to region, depositing only as much monomer as necessary at each stop. Typical dispensers include a micropipette to deliver the monomer **solution** to the substrate and a robotic system to control the position of the micropipette with respect to the substrate, or an ink-jet printer. In other embodiments, the dispenser includes a series of tubes, a manifold, an array of pipettes, or the like so that various reagents can be delivered to the reaction regions simultaneously.

Detailed Description Text - DETX (41):

Each tray is filled with a particular reagent for coupling in a particular chemical reaction on an individual pin. Accordingly, the trays will often contain different reagents. Since the chemistry disclosed herein has been

established such that a relatively similar set of reaction conditions may be utilized to perform each of the reactions, it becomes possible to conduct multiple chemical coupling steps simultaneously. In the first step of the process the invention provides for the use of substrate(s) on which the chemical coupling steps are conducted. The substrate is optionally provided with a spacer having active sites. In the particular case of oligonucleotides, for example, the spacer may be selected from a wide variety of molecules which can be used in organic environments associated with synthesis as well as **aqueous** environments associated with binding studies. Examples of suitable spacers are polyethyleneglycols, dicarboxylic acids, polyamines and alkylenes, substituted with, for example, methoxy and ethoxy groups. Additionally, the spacers will have an active site on the distal end. The active sites are optionally protected initially by protecting groups. Among a wide variety of protecting groups which are useful are FMOC, BOC, t-butyl esters, t-butyl ethers, and the like. Various exemplary protecting groups are described in, for example, Atherton et al., Solid Phase Peptide Synthesis, IRL Press (1989), incorporated herein by reference. In some embodiments, the spacer may provide for a cleavable function by way of, for example, exposure to acid or base.

Detailed Description Text - DETX (43):

Yet another method which is useful for synthesis of polymers and small ligand molecules on a solid **support** "bead based synthesis." A general approach for bead based synthesis is described copending application Ser. No. 07/762,522 (filed Sep. 18, 1991); Ser. No. 07/946,239 (filed Sep. 16, 1992); Ser. No. 08/146,886 (filed Nov. 2, 1993); Ser. No. 07/876,792 (filed Apr. 29, 1992); PCT/US94/12347 (filed Nov. 2, 1994) and PCT/US93/04145 (filed Apr. 28, 1993), the disclosures of which are incorporated herein by reference.

Detailed Description Text - DETX (49):

The advent of methods for the synthesis of diverse chemical compounds on solid **supports** has resulted in the genesis of a multitude of diagnostic applications for such chemical libraries. A number of these diagnostic applications involve contacting a sample with a solid **support,** or chip, having multiple attached biological polymers such as peptides and oligonucleotides, or other small ligand molecules synthesized from building blocks in a stepwise fashion, in order to identify any species which specifically binds to one or more of the attached polymers or small ligand molecules.

Detailed Description Text - DETX (51):

A number of factors contribute to the successful synthesis and use of oligomer arrays on solid **supports**. For example, issues of relevance to the use of derivatized glass substrates for carrying out VLSIPS.TM. synthesis of

peptide arrays are the spacing of the synthesis initiation sites, the wettability of the surface by organic solvents and **aqueous solutions,** and the extent to which non-specific binding of receptors, antibodies or other biological macromolecules occurs.

Detailed Description Text - DETX (55):

The derivatization of **supports** for the preparation of ligand arrays, as well as other forms of solid phase synthesis, must take into account several issues relating to both the synthesis which occurs on the **support** and the subsequent use of the arrays in binding studies and diagnostic assays. Foremost among the many issues are the spacing of initiation sites, the wettability of the surface by both organic solvents and **aqueous solutions,** and the extent to which non-specific binding of receptors occurs.

Detailed Description Text - DETX (56):

The spacing of synthesis initiation sites on a solid **support** can affect not only the synthesis of the ligand array but also the binding events between a receptor and a tethered ligand. The synthesis can be influenced through phenomena such as free radical formation during photolytic reaction (in light-directed synthesis), solvent accessibility and surface electrostatic effects.

Detailed Description Text - DETX (57):

The wettability of the **support,** or substrate surface, is also likely to have a direct influence on the yield of coupling reactions and subsequent binding events. The presentation of peptides or other ligands for recognition is expected to be a function of not only the hydrophobicity/hydrophilicity of the peptide or ligand, but also the physicochemical nature of the surface to which it is attached. Thus, hydrophilic peptide sequences are expected to extend fully into the surrounding **aqueous** environment, thereby maximizing their availability for recognition and binding by receptors. In contrast, hydrophobic sequences in the presence of a moderately hydrophobic substrate surface can collapse onto the surface and effectively be eliminated from the pool of available ligands presented to a receptor.

Detailed Description Text - DETX (58):

In view of the above considerations, the present invention provides a method for affixing functional sites to the surface of a solid substrate at a preselected density. In this method, a solid substrate is reacted with a derivatization reagent having a substrate attaching group on one end and a reactive site on a distal end (away from the surface) to provide a substrate having an even distribution of reactive sites. The derivatized substrate is

then contacted with a mixture of linking molecules and diluent molecules. The linking molecules each have reactive groups which are capable of **covalent** attachment to the reactive sites on the derivatized substrate. The linking molecules additionally have a functional group which is optionally protected. The ratio of the linking molecules to the diluent molecules in the mixture is selected to control the functional site density on the surface of the substrate. The contact is carried out for a sufficient period of time to bind the linking molecules and the diluent molecules to the substrate.

Detailed Description Text - DETX (59):

The broad concept of this aspect of the invention is provided in FIG. 1 which illustrates the "doped process" of derivatizing a solid **support**. A related method, termed the "standard process," is provided for comparison in FIG. 2. In the doped process, a **support** (for example, a glass slide) is cleaned and derivatized with an aminoalkylsilane to provide a surface of amine functional groups. Treatment of this derivatized substrate with a mixture of linking molecules (for example, NVOC-aminocaproic acid) and diluent molecules (for example, protected amino acids) provides a surface having synthesis initiation sites at a preselected density. The density of synthesis initiation sites will depend on the particular ratio of linking molecules to diluent molecules which is used.

Detailed Description Text - DETX (64):

The linking molecules used in the present invention are preferably of sufficient length to permit any polymers synthesized thereon to interact freely with molecules exposed to the polymers. The linking molecules should be 3-50 atoms long to provide sufficient exposure of ligands to their receptors. Typically, the linking molecules will be aryl acetylene, ethylene glycol oligomers containing 2-14 monomer units, diamines, diacids, amino acids, peptides, or combinations thereof. In some embodiments, the linking molecule can be a polynucleotide. The particular linking molecule used can be selected based upon its hydrophilic/hydrophobic properties to improve presentation of the polymer synthesized thereon to certain receptors, proteins or drugs. As noted above, the linking molecule, prior to attachment to the derivatized surface has an appropriate functional group at each end, one group appropriate for attachment to the reactive sites on a derivatized surface and the other group appropriate as a synthesis initiation site. For example, groups appropriate for attachment to the derivatized surface would include amino, hydroxy, thiol, carboxylic acid, ester, amide, isocyanate and isothiocyanate. Additionally, for subsequent use in synthesis of polymer arrays or libraries, the linking molecules used herein will typically have a protecting group attached to the functional group on the distal or terminal end of the linking

molecule (opposite the solid **support**).

Detailed Description Text - DETX (69):

The present invention also provides solid **supports** which are derivatized to provide acidic surfaces, or "carboxy chips." The carboxy chips can be considered as "reverse polarity" surfaces (as compared with the more typical aminopropylsilane derivatized surfaces). Such reverse polarity surfaces will find application in combinatorial synthesis strategies which require a carboxylic acid initiation site. For example, peptide synthesis which is carried out from the N-terminal end to the C-terminal end can be carried out on a carboxy chip. Additionally, small molecules such as prostaglandins, .beta.-turn mimetics and benzodiazepines can also be synthesized on a carboxy chip. Carboxy chips will also find application in the preparation of chips having synthesis initiation sites which are amines. In this aspect, the carboxy chips will be reacted with a suitably protected alkylenediamine to generate an amino surface.

Detailed Description Text - DETX (70):

Carboxy chips can be prepared by a variety of methods. In one group of embodiments, a solid **support** is derivatized with an aminoalkylsilane to provide a surface of attached amino groups. The derivatized surface is then treated with an anhydride such as glutaric anhydride to acylate the amino group and provide a surface of carboxylic acid functionalities. In other embodiments, the aminoalkylsilane is first reacted with an anhydride (i.e., glutaric anhydride) to generate a carboxylic acid silane which can then be coupled to the solid **support,** and similarly provide a surface of carboxylic acid residues.

Detailed Description Text - DETX (72):

The present invention also provides a method of preparing surfaces in which a polymer, having synthesis properties similar to a commercial peptide resin is attached to a solid **support**. The polymer films provide a porous three-dimensional matrix functionalized with reactive groups that serve as starting points for oligonucleotide or peptide synthesis. One of the potential advantages of these films in VLSIPS.TM. applications is that they may provide a much larger number of synthesis sites per unit area of substrate than is offered by the current generation of monofunctional silane-derivatized glass surfaces, while maintaining a similar or greater spacing between sites. Additionally, the use of an organic polymer on a solid surface will provide greater solvent compatibility and flexibility of the reaction site for attachment of the synthesis building blocks. Another advantage is the potential improvement in surfaces for performing bioassays which results from the variety of polymers available and the degrees of polymer porosity which can

be obtained. The extent of binding of target molecules (receptors) to the immobilized oligonucleotide or peptide sequences (ligands) may be substantially increased, which enhances detection, and the multiplicity of binding sites within the polymer **support** may provide additional kinetic enhancement. Thus surfaces can be designed and prepared for optimum properties in a particular assay. This optimization will take advantage of the relatively thick but loosely woven polymer network that allows macromolecules to diffuse in and out of a layer of tethered ligands. Still other potential advantages that may be achieved with polymer-coated surfaces prepared by the present methods involve improved processing for reusing the surface, easier characterization of the surface for quality control in synthesis, and reduction of potential problems associated with the use of glass surfaces.

Detailed Description Text - DETX (73):

A variety of approaches can be employed for the preparation of polymer-coated solid surfaces. In one approach, the solid surface used is a rigid polymer which is then crosslinked with a "soft" polymer layer to confer desired surface properties. Alternatively, a solid surface such as a glass slide can be coated with a polymer film to form a **composite**. These **composites** can be created by covalently crosslinking the polymer to silanized glass, by in situ polymerization of monomers on a silanized glass surface, or by relying on the mechanical strength of a polymer film to completely wrap and adhere to a slide that has been dipped.

Detailed Description Text - DETX (76):

The polymers which are used to coat the solid **support** can also be selected based upon their functional groups which will serve as synthesis initiation sites. Typically, polymers having primary amine, carboxyl or hydroxyl functional groups will be selected.

Detailed Description Text - DETX (77):

Polymers having primary amine functional groups are of interest as these polymers can be readily adapted to coupling chemistry currently used in the VLSIPS.TM. process. Suitable polymers having primary amine functional groups include **polyethyleneimine** (linear or branched polymers, see Royer, G., Chemtech, pp. 694-700 (November 1974); Narayanan, S., et al., Anal. Biochem. 188:279-284 (1990); Rainsden, H., U.S. Pat. No. 4,540,486 (1995); Watanabe, K., et al., Anal. Biochem. 127:155-158 (1982); Meyers, W. et al., J. Amer. Chem. Soc. 99:6141-6142 (1977); Royer, G., et al., J. Macromolec. Sci. Chem. A10:289-307 (1976); Chao, K. et al, Biotechnology and Bioengineering 28:1289-1293 (1986)); polyacrylamide (see Inman, J., et al. Biochemistry 8:4074 (1967)); and polyallylamine which are all commercially available (Aldrich

Chemical Company, St. Louis, Mo., U.S.A.; Polyscience, location; and Dow Chemical Company, Midland, Mich., U.S.A.). Other polymers, such as polydimethylacrylamide, can be synthesized according to published procedures (see Atherton, E., et al. in Solid Phase Peptide Synthesis: A Practical Approach, Chapter 4, pp. 39-45, IRL Press (1989); and Arshady, R., et al., J. Chem. Soc. Perkin. Trans. 1:529 (1981)). Structures for these polymers are provided in FIG. 3. Additionally, these polymers are soluble in polar solvents such as water, methanol and DMF.

Detailed Description Text - DETX (79):

Polymers having hydroxyl functional groups are also useful as the resulting surfaces are extremely wettable. Examples of suitable polymers include polyethyleneglycol (PEG, see Rapp Polymere Catalogue, Harris, J., J. Polym. Sci. Polym. Chem. Ed. 22:341 (1984); and Pillai, V., et al., J. Org. Chem. 45:5364-5370 (1980)); poly(vinyl **alcohol**); and carbohydrates (see J. Chemical Society Chem. Comm., p. 1526 (1990)) which are shown in FIG. 5. Solid **supports** coated with carbohydrate polymers or glycans are discussed in Section VI, below.

Detailed Description Text - DETX (80):

The preparation of thin polymers films on solid surfaces can be accomplished using a variety of methods including dip coating, **covalent** attachment and in situ polymerization (see FIG. 6).

Detailed Description Text - DETX (82):

Films can be created on solid substrates by dip coating with the polymer **solution,** followed by evaporation of the solvent and stabilization of the coating using crosslinking agents or UV treatment. Suitable crosslinking agents will depend on the nature of the functional groups present in the polymer. For polymers having primary amine functional groups, crosslinking agents such as glutaraldehyde or Xama (a polyfunctional aziridine: see, Watanabe, et al., Anal. Biochem. 127:155-158 (1982)) are preferred. Crosslinking agents which are useful for other polymers are known to those of skill in the art. Additionally, the degree of polymer crosslinking can be varied to produce films which are optically transparent and of uniform thickness. A related method of producing a uniform thickness polymer coating utilizes a spin-coating technique. FIG. 7 illustrates a typical reaction scheme for the preparation of **polyethyleneimine**-coated glass substrates.

Detailed Description Text - DETX (83):

Covalent Attachment

Detailed Description Text - DETX (84):

Another method for the preparation of polymer films on solid surfaces involves preparation of the selected polymer followed by **covalent** attachment of the polymer to functional groups which are present on the substrate surface or modified substrate surface. In one embodiment, a glass surface is cleaned and silanized using an aminopropyltriethoxysilane to provide a glass surface having primary amine functional groups. The amine functional groups can then be reacted with crosslinking groups (such as glutaraldehyde) and treated with **solutions** of an appropriate polymer (see FIG. 8). Alternatively, glass surfaces which have been modified with aminopropylsilanes can be reacted with polymers having carboxylic acid functional groups (using, for example, water soluble carbodiimides) or by direct reaction of the modified surface with a polymer having attached anhydride groups (see FIG. 9). In other embodiments, glass surfaces can be silanized with silanes having epoxide functional groups and subsequently reacted directly with polymers having either amine or hydroxy functional groups (see FIGS. 10 and 11). In addition, glass surfaces can be planarized by using polysiloxanes known as "spin on glasses" which may provide both a more uniform planar surface and/or a substantially higher density of functional sites which further provides for better subsequent derivatization.

Detailed Description Text - DETX (87):

Still other methods of preparing polymer-coated solid **supports** will use combinations of two or more of the above methods. For example, a polymer film can be "grafted" onto a glass **support** by first silanizing the glass with an acrylamido-alkyl trialkoxysilane. Subsequent polymerization of an acrylamide copolymer layer on top of the silanized glass provides a covalently attached film which exhibits excellent resistance to all of the conditions used for oligonucleotide synthesis, deprotection and hybridization.

Detailed Description Text - DETX (88):

The polymer-coated **support** can be tailored to provide optimal properties for synthesis and for biological assays. For example, the final concentration of functional groups (amine or hydroxyl) in the polymer can be controlled by varying the relative amounts of nonfunctionalized and functionalized monomers used in forming the polymer. Additionally, the porosity and solubility of the polymer films can be controlled by varying the concentrations of monomers and crosslinking agents in the composition. Thus, a high degree of crosslinking gives a rigid insoluble polymer with low pore size, whereas omitting the crosslinking agent altogether will result in soluble linear polymer chains (with functional groups) extending off the surface of the substrate from the attachment sites.

Detailed Description Text - DETX (93):

In still another aspect, the present invention provides solid surfaces which are coated with a layer of high molecular weight (500 Kd) dextran (.alpha.1-6 poly D-glucose). Solid surfaces which are coated with dextran or other glycans provide more hydrophilic surfaces which exhibit improved characteristics for monitoring the binding of a receptor to a **support**-bound ligand.

Detailed Description Text - DETX (94):

In general, the glycan-coated surfaces can be prepared in a manner analogous to the preparation of polymer-coated surfaces using **covalent** attachment. Thus, a glass surface can be modified (silanized) with reagents such as aminopropyltriethoxysilane to provide a glass surface having attached functional groups (in this case, aminopropyl groups). The modified surface is then treated with a **solution** of a modified dextran to provide a surface having a layer of dextran which is covalently attached.

Detailed Description Text - DETX (95):

The method of covalently attaching a dextran or other carbohydrate to the glass surface can be carried out using a variety of chemical manipulations which are well known to those of skill in the art. In one embodiment, the surface is modified to produce a glass surface having attached primary amine groups using reagents such as aminopropyltriethoxysilane. The resulting amines are then reacted (using water soluble carbodiimides) with dextrans which have been previously modified with carboxymethyl groups. In another embodiment, the glass surface is modified with hydroxy groups using reagents such as hydroxypropyltriethoxysilane. Subsequent reaction of the hydroxy moiety with epichlorohydrin provides a surface having attached epoxide functional groups. The epoxides can then be reacted directly with hydroxyl groups present in dextran to provide **covalent** attachment of the dextran to the modified surface.

Detailed Description Text - DETX (96):

Following **covalent** attachment of the dextran to the glass surface, the carbohydrate can be further derivatized to provide synthesis initiation sites for peptide, oligonucleotide or other small molecule synthesis. For example, treatment of dextran-modified surfaces with bromoacetic acid results in derivatives having attached carboxymethyl groups. The carboxylic acid groups can be used as synthesis initiation sites or they can be further modified with lower diaminoalkanes to provide primary amines as synthesis initiation sites. See, Cass, et al., In PEPTIDES: CHEMISTRY, STRUCTURE AND BIOLOGY, Hodges, et
al., eds., ESCOM, Leiden pp. 975-977 (1994).

Detailed Description Text - DETX (100):

In one group of embodiments, used ligand arrays are treated with a **solution** of a chaotropic reagent to remove any bound receptors. The resulting arrays can be used directly in another assay or the array can be placed in a storage **solution** which retards degradation of the ligand array. The chaotropic agents which are useful in the present method are selected depending upon the receptor which is to be removed from the surface. In preferred embodiments, the chaotropic reagent is a member selected from the group consisting of guandidine hydrochloride, urea, glycine, Tris and guanidine with dithiothreitol. For ligand arrays of peptides to which a protein is bound, the preferred chaotropic agents are guanidine hydrochloride, urea, or glycine hydrochloride pH 2.0. For removal of tightly bound receptors such as antibodies, a mixture of guanidine and dithiothreitol is preferred. For removal of DNA which is bound to another oligonucleotide ligand, the preferred chaotropic reagent is 10 mM Tris, 0.1 mM EDTA.

Detailed Description Text - DETX (101):

In another group of embodiments, the cleaned and regenerated ligand array is placed into a storage **solution**.

Detailed Description Text - DETX (102):

The present inventive method can be applied to a wide variety of solid supported ligand arrays including those which are formed according to any of the methods described in the above General Methods section. Additionally, the present method of surface regeneration can be used for any of the ligand arrays formed on the polymer-coated solid **supports,** also discussed above. In preferred embodiments, the used ligand array is a VLSIPS.TM. chip.

Detailed Description Text - DETX (105):

The present invention also provides methods for the synthesis of oligomers on a solid **support**. General methods for the synthesis of oligomers on solid **supports** have been described above. Thus, the present invention provides methods for the synthesis of oligomers on a solid **support** wherein the protecting groups on the monomers used in the oligomer preparation are exchanged following addition of the monomer to the growing oligomer. This is illustrated in FIGS. 16 and 17 for the preparation of oligonucleotides and peptides, respectively. As shown in FIG. 16, a solid **support** having preselected regions is first constructed which has attached photolabile protecting groups in each of the preselected regions. Using photolithographic techniques described in the above-noted General Methods section, the photolabile protecting groups can be removed in one preselected area and a monomer bearing a chemically-removable protecting group is attached. Standard,

chemically-removable protecting groups include those groups which are commercially available and which are known to be removable under typical chemical conditions. Examples of such protecting groups include FMOC, DMT, BOC, t-butyl esters and t-butyl ethers. Following the attachment of such a protected monomer, the protecting group is removed under conditions described in, for example, Greene, et al., Protective Groups In Organic Chemistry, 2nd Ed., John Wiley & Sons, New York, N.Y., 1991, previously incorporated herein by reference. The reactive functionality which was previously protected with the chemically-removable protecting group is then re-protected with a photolabile protecting group, using, for example, a derivative of the formula:

Detailed Description Text - DETX (109):

In still another group of embodiments, all preselected areas are derivatized with a first monomer, each of the monomers having a chemically-removable protecting group. Following the addition of the first monomer to each of the preselected regions, the protecting groups are all removed in a single step using chemical deprotection in the form of a wash across the solid **support**. Reprotection of each of the growing oligomers with a photolabile protecting group is then carried out in the form of another wash across the entire solid **support**. Following this reprotection, photolithographic techniques of oligomer synthesis can be continued using monomers having chemically-removable protecting groups.

Detailed Description Text - DETX (116):

The standard process of surface derivatization is described below with reference to the illustration presented in FIG. 2. Glass microscope slides are cleaned by treatment with a Nochromix/sulfuric acid cleaning **solution**. The slides are then etched with 10% NaOH for three minutes at 70.degree. C., rinsed with 1% HCl, and finally rinsed with ethanol to provide a substrate having exposed hydroxyl groups. The resulting clean glass substrates are then treated with a 1% silane **solution** (1:10 mole ratio of 3-aminopropyltriethoxysilane:methyltriethoxysilane) in dichloromethane for 15 minutes. After standing at room temperature for 30 minutes the substrates are then cured at 100.degree. C. for 15 minutes to provide aminopropyl silylated substrates. The amino groups which are present are then acylated with NVOC-aminocaproic acid, using standard BOP coupling techniques. After 2 hours, any unreacted amino groups are capped as their acetamides using acetic anhydride to provide a derivatized surface having attached NVOC-aminocaproic acid spacing groups.

Detailed Description Text - DETX (118):

The doped process of surface derivatization is described below with

reference to the illustration presented in FIG. 1. A glass microscope slide is cleaned as described above in the standard process. The resulting clean glass substrate is treated with a 1% silane **solution** (aminopropyltriethoxysilane in dichloromethane) for 15 minutes. After standing at room temperature for 30 minutes, the substrate is cured at 100.degree. C. for 15 minutes. To the aminopropyl silylated substrate is added a diluent mixture of NVOC-aminocaproic acid and a suitably protected amino acid (presented as "R" in FIG. 1). The species in the mixture are coupled to the amino group now present on the substrate using BOP chemistry. After 2 hours, any remaining amino groups present on the substrate are capped using acetic anhydride to provide a surface having a predetermined density of linking groups per unit area.

Detailed Description Text - DETX (122):

The density of NVOC-protected amine on a derivatized surface was assessed using the NVOC photoproduct fluorescence assay. Substrates were prepared according to the doped process in Example 1 using either acetylated glycine or serine to dilute the number of NVOC-aminocaproic acid linking groups being coupled to a glass surface having attached aminopropylsilanes. The surfaces were labeled with a 39:1 mixture of phenylisothiocyanate to fluorescein isothiocyanate in the labeling **solution** (10 mM total isothiocyanate in 1% DIEA/NMP was used in order to minimize fluorescence quenching effects). For photolysis, a solvent of 5 mM H.sub.2 SO.sub.4 in dioxane was used with illumination at 365 nm and at about 10 mW/cm.sup.2 for up to 12 minutes. After photolysis of the NVOC-protected surfaces and collection of the solvent used, fluorescence emission was measured at 400 nm in a spectrofluorimeter following a 330 nm excitation. The results are presented in FIG. 13. The linear relationship observed for the glycine-doped series implies that the rates of coupling for the two components with the surface are similar. The nonlinear but reproducible relationship observed for the serine-doped surfaces, although not fully understood, implies either that the components react with the aminopropylsilane surface with different rates, or that some loss of the FMOC group occurs under the reaction conditions, resulting in enhanced coupling of the NVOC-protected linker at the higher serine-to-linker ratios. However, the reproducibility of the curve implies that the approach may still be used to control site density. Comparison of the fluorescence signals to a calibration curve generated from known concentrations of NVOC-glycine in **solution** allows for an estimate of site spacing on a surface to be made. Table 1 summarizes these results.

Detailed Description Text - DETX (132):

This example illustrates the relative amounts of specific and non-specific binding which can be achieved between a substrate on a solid **support** and a

receptor utilizing a variety of linking groups.

Detailed Description Text - DETX (134):

Several slides with differing surfaces were prepared as above and were then subjected to photolysis in order to remove the terminal NVOC group. Biotin was coupled onto the liberated amino group via BOP/HOBt activation of the acid functionality of the biotin molecule, to provide biotinylated surfaces in which the nature of the linker joining the biotin to the surface differed. Eight different surfaces were prepared: one labeled "standard aminocaproic" which is described above as "standard process" and seven other surfaces in which the ratio of linker to diluent was varied as described above as "doped process". The diluent molecule was either N-acetyl glycine or N-acetyl serine and the ratios tested were either 100% linker/0% diluent or 10% linker/90% diluent. A template was clamped onto the surface of each slide which segregated the surface into 3 individual wells. A Buna-N gasket was used to seal the template to the surface. Commercially available fluorescein-labeled strepavidin and strepavidin (Molecular Probes Inc., Eugene, Oreg., U.S.A.) were used as a mixture in order to minimize fluorescence self quenching effects. Surface-bound fluorescence was measured via confocal fluorescence microscopy employing the excitation and emission wavelengths of fluorescein. Control experiments (data not shown) demonstrated that using ratios of labeled strepavidin to strepavidin of 0.05-1:1 respectively, resulted in little or no fluorescence self quenching being observed. Control experiments (data not shown) also demonstrated that 2 hr incubation times were sufficient to achieve equilibrium between the surface-bound biotin and **solution**-strepavidin.

Detailed Description Text - DETX (135):

Following incubation of the surface with the mixture of labeled strepavidin/unlabeled strepavidin in PBS/Tween-20 buffer for 2 hr, the wells were washed with buffer and surface-bound fluorescence was measured. Non-specific binding of strepavidin to the surface was measured by preincubation the strepavidin mixture with excess biotin (in **solution**) to block the binding sites, followed by incubation of the blocked strepavidin with the surfaces as before. Non-specific binding was subtracted from the measurements determined above to give a net binding signal, which is illustrated in FIG. 15. Larger net binding signals are indicative of greater discrimination between specific and non-specific binding. As is observed from the graph, the surface prepared from 15-ATOM-PEG exhibited the greatest discrimination between specific and non-specific binding.

Detailed Description Text - DETX (139):

To a **solution** of aminopropyltriethoxysilane (3.0 g, 13.55 mmol, Petrarch

Systems, Bristol, Pa.) in 35 mL of CH.sub.2 Cl.sub.2 was added glutaric anhydride (1.54 g, 13.50 mmol) at room temperature. The reaction mixture was warmed slightly upon addition of the anhydride. After stirring for 2 hours, the solvent was removed under reduced pressure to yield 4.5 g of a colorless oil. NMR analysis indicated that the product was 95% pure and no further purification was performed.

Detailed Description Text - DETX (141):

Glass microscope slides were derivatized as described previously under the "standard process". Thus a 1% **solution** of 1:10 N-(triethoxysilylpropyl)glutaramide: methyltriethoxysilane in CH.sub.2 Cl.sub.2 was prepared and freshly cleaned microscope slides were immersed into the **solution** for 15 minutes. The slides were briefly rinsed with CH.sub.2 Cl.sub.2 and allowed to stand at room temperature for 30 minutes, then cured at 100.degree. C. for 15 minutes.

Detailed Description Text - DETX (143):

The derivatized slide from above is immersed into a **solution** of N-(t-butoxycarbonyl)ethylenediamine (Fluka Chemie, Switzerland) (0.1M) and 1,3-diisopropylcarbodimide (0.1M) in DMF for 2 hours at room temperature. The slide is extensively washed with DMF, CH.sub.2 Cl.sub.2, and MeOH and then air-dried. Residual carboxylic acid groups are blocked by incubating the slide with excess diazomethane in ether for 10 minutes at room temperature. After washing the slide with CH.sub.2 Cl.sub.2, the BOC group is removed by immersing the slide in 50% TFA/CH.sub.2 Cl.sub.2 for 30 minutes, and washed again with CH.sub.2 Cl.sub.2. The slide is then immersed in a 5% **solution** of DIEA/DMF to neutralize the TFA salts and is further derivatized by immersion in a 0.1M **solution** of FMOC-15-ATOM-PEG (See Example 4) in DMF activated as its OBt ester via BOP/HOBt. After standing at room temperature for 2 hours, the slide is washed with DMF, CH.sub.2 Cl.sub.2, and MeOH and then air dried.

Detailed Description Text - DETX (147):

To a **solution** of acryloyl chloride (8.5 mL, 105 mmol) in dry dichloromethane (250 mL) cooled to 0.degree. C. was added dropwise a mixture of 3-aminopropyltriethoxysilane (23.5 mL, 100 mmol) and triethylamine (13.9 mL, 100 mmol) in dichloromethane (50 mL). After completion of addition the reaction mixture was stirred for 30 min, then filtered. The filtrate was concentrated to an oil, diluted with hexane (100 mL) and filtered. The resulting filtrate was concentrated to oil and distilled in vacuo. The product was obtained as a viscous liquid (21.2 g, 77%), b.p. 142-145/1 mm Hg.

Detailed Description Text - DETX (149):

To a **solution** of acryloyl chloride (2.25 mL, 65 mmol) in ethyl ether (200 mL) cooled to 0.degree. C. was added ethylenediamine (4.2 mL, 63 mmol) in ether (25 mL) slowly with vigorous stirring. After the addition was completed, the product (2-aminoethyl)acrylamide hydrochloride was removed by filtration, washed with ether on the filter and dried to provide 8.2 g (87%) of the product.

Detailed Description Text - DETX (150):
(c) Preparation of polymer-coated glass **supports**.

Detailed Description Text - DETX (152):
The second glass plate of the same surface quality was treated for 15-20 min with 5-10% **solution** of 3-(triethoxysilyl)propylacrylamide in 95% **alcohol**. After this it was washed intensively with **alcohol** and dried by nitrogen stream. This glass was used further as bound glass. An **aqueous** monomer **solution** was prepared providing 0.8M N,N-dimethylacrylamide, 0.1M (2-aminoethyl)acrylamide, 16 mM methylene-bisacrylamide and a small amount of ammonium persulfate **solution**. Before using, the mixture was filtered.

Detailed Description Text - DETX (153):
Two 13 mm (or other size) spacer strips were put on sides of cover glass. The monomer **solution** was activated by a trace amount of TEMED, and the polymerizing mixture was then put on the surface of cover glass between spacers and covered with bound glass. The glass "sandwich" was fixed with two clamps and kept for 15-20 min. After completion of polymerization, the glass "sandwich" was rinsed with distilled water and carefully disconnected. The resulting gel chip was washed with distilled water for 5-10 hours to remove any low molecular weight compounds, then with 0.1M KOH for 5 min, and with distilled water for 5 min. After washing with **alcohol,** the chips were dried.

Detailed Description Text - DETX (155):
This example illustrates a method for the attachment of a thin film of crosslinked amino-functionalized acrylamide copolymer to a glass **support** for use in solid-phase oligonucleotide synthesis. In this example, acrylamide groups are attached to the glass **support** using acrylamidopropyltriethoxysilane. A synthesis initiation site is introduced into the polymer backbone by polymerizing N-(2-aminoethyl)acrylamide in an **aqueous solution** of dimethylacrylamide. Other monomers can also be used (e.g., N-(2-hydroxyethyl)-acrylamide), and oligonucleotide synthesis proceeds smoothly with either amino- or hydroxyl-functionalized **supports**. Polymerization was carried out between the acrylamide-derivatized substrate and another glass plate which was prepared so as not to adhere to the polymer film afterwards

(see Example 6).

Detailed Description Text - DETX (162):
Dextran T500 (1.5 g having a molecular weight 500,000, available from Pharmacia Biotech, Inc., Piscataway, N.J., U.S.A.) was dissolved in **aqueous** base (2N NaOH) and bromoacetic acid (1.0 g) was added. After 3 hr at room temperature, an additional 1.0 g of bromoacetic acid was added and the mixture was kept at room temperature for 24 hr. The mixture was then dialyzed against distilled water for 24 hr, during which time the water was changed several times. Following dialysis, the **aqueous solution** was lyophilized to provide carboxymethyldextran as a solid (1.9 g, corresponding to approximately 24% of all secondary hydroxyls being carboxymethylated).

Detailed Description Text - DETX (164):
This example illustrates the coupling of carboxymethyldextran to a modified solid **support**.

Claims Text - CLTX (1):
1. A method of preparing a polymer-coated **support** for use in solid-phase synthesis using in situ polymerization, comprising:

Claims Text - CLTX (3):
(b) contacting a **support** derivatized with a derivatizing agent having a substrate attaching group attached to the **support** and a reactive site distal to the **support** with said mixture to form a polymer linked to the **support** via the reactive site on the derivatizing agent.

Claims Text - CLTX (5):
(c) functionalizing said polymer-coated **support** to provide functional groups as synthesis initiation sites.

Claims Text - CLTX (7):
4. The method of claim 1, wherein the **support** is glass.

Claims Text - CLTX (9):
6. The method of claim 1, wherein the polymer formed on the **support** has amine, carboxyl and/or hydroxyl functional groups.

Claims Text - CLTX (10):
7. The method of claim 6, wherein the polymer is selected from the group consisting of **polyethyleneimine,** polyethyleneglycol, polyvinyl **alcohol,** and carbohydrates.

Other Reference Publication - OREF (15):

Rajasekhara Pillai, V.N. et al., "New, Easily Removable Poly(ethyleneglycol) **Supports** for the Liquid-Phase Method of Peptide Synthesis," J. Org. Chem. 45(26):5364-5370 (1980).

EAST Search History

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	11	"5753008"	USPAT; EPO; DERWENT	AND	ON	2006/04/25 19:57
L2	21	"5049282"	USPAT; EPO; DERWENT	AND	ON	2006/04/25 18:21
L3	1	l1 and covalent	USPAT; EPO; DERWENT	AND	ON	2006/04/25 18:25
L4	5	2 and covalent	USPAT; EPO; DERWENT	AND	ON	2006/04/25 19:21
L5	1	1 and covalent bond	USPAT; EPO; DERWENT	AND	ON	2006/04/25 19:21
L6	15	diaminohexane same propanol and membrane	USPAT; EPO; DERWENT	AND	ON	2006/04/25 19:58
L7	12	6 and support	USPAT; EPO; DERWENT	AND	ON	2006/04/25 20:00
L8	0	210/500.37 and 6	USPAT; EPO; DERWENT	AND	ON	2006/04/25 20:01
L9	2	210/500.37 and diaminohexane	USPAT; EPO; DERWENT	AND	ON	2006/04/25 20:05
L10	0	9 and alcohol	USPAT; EPO; DERWENT	AND	ON	2006/04/25 20:02
L11	0	9 and covalent	USPAT; EPO; DERWENT	AND	ON	2006/04/25 20:04
L12	29	diaminohexane same alcohol and membrane	USPAT; EPO; DERWENT	AND	ON	2006/04/25 20:05
L13	3	12 and polyimide	USPAT; EPO; DERWENT	AND	ON	2006/04/25 20:05
L14	0	13 and covalent	USPAT; EPO; DERWENT	AND	ON	2006/04/25 20:06
L15	3	13 and heating	USPAT; EPO; DERWENT	AND	ON	2006/04/25 20:06

EAST Search History

L16	1	15 and support	USPAT; EPO; DERWENT	AND	ON	2006/04/25 20:12
L17	3	12 and composite	USPAT; EPO; DERWENT	AND	ON	2006/04/25 20:14
L18	0	210/500.38 and 12	USPAT; EPO; DERWENT	AND	ON	2006/04/25 20:15
L19	0	210/500.38 and diaminohexane same propanol	USPAT; EPO; DERWENT	AND	ON	2006/04/25 20:16
L20	0	210/490 and diaminohexane same propanol	USPAT; EPO; DERWENT	AND	ON	2006/04/25 20:17
L21	15	diaminohexane same propanol and membrane	USPAT; EPO; DERWENT	AND	ON	2006/04/25 20:19
L22	0	polyethyeneimine same water and propanol	USPAT; EPO; DERWENT	AND	ON	2006/04/25 20:20
L23	0	polyethyeneimine and aquous solution and alcohol and membrane	USPAT; EPO; DERWENT	AND	ON	2006/04/25 20:21
L24	8	polyethyleneimine and aquous solution and alcohol and membrane	USPAT; EPO; DERWENT	AND	ON	2006/04/25 20:21
L25	1971	polyethyleneimine and aqueous solution and alcohol and membrane	USPAT; EPO; DERWENT	AND	ON	2006/04/25 20:21
L26	582	25 and covalent	USPAT; EPO; DERWENT	AND	ON	2006/04/25 20:22
L27	164	26 and composite	USPAT; EPO; DERWENT	AND	ON	2006/04/25 20:22
L28	24	27 and polyimide and support	USPAT; EPO; DERWENT	AND	ON	2006/04/25 20:22